Supporting Information

Nanoscale Metal–Organic Framework for Combined Photodynamic and Starvation Therapy in Treating Breast Tumors

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Content

1. Materials and Instrumentations

All reactants were reagent grade and were used as purchased without further purification. 2,4-Dimethyl-1*H*-pyrrole, *N*,*N*-diisopropylethylamine, boron trifluoride ethyl ether complex ($BF_3 \cdot Et_2O$), iodine, iodic acid, and 2-hydroxyterephthalic acid were purchased from Aladdin Reagent Co., Ltd. 6-Bromohexanoyl chloride and 1,3-diphenylisobenzofuran (DPBF) were purchased from TCI (Shanghai) Development Co., Ltd. Acetic acid was purchased from Sinopharm Chemical Reagent Co., Ltd. $HfCl_4$, and KI were purchased from Macklin Inc. MnCO (CAS# 1001015-18-4) was purchased from MedChemExpress Co. Ltd. All organic solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. Dehydrated solvents were obtained after treating solvents with standard procedures. Ultra-pure water was prepared with an Aquapro System (18 MΩ).

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (Shanghai) Trading Co. Ltd. 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA), chlorpromazine hydrochloride (CPZ), methyl-β-cyclodextrin (MβCD), and amiloride hydrochloride (AMR) were purchased from MedChemExpress Co. Ltd. Calcein-AM/PI Double Stain Kit was purchased from Yeasen Biotech (Shanghai) Co., Ltd. Singlet Oxygen Sensor Green (SOSG), and JC-1 were purchased from Thermo Fisher Scientific Inc. Lactate Assay Kit was purchased from Nanjing Jiancheng Bioengineering Institute. ATP Assay Kit was purchased from Beyotime Biotech (Shanghai) Inc.

Phosphate-Buffered Saline (PBS), Dulbecco's Phosphate-Buffered Saline (DPBS), and Fetal Bovine Serum (FBS) were purchased from Biological Industries USA, Inc. Dulbecco's Modified Eagle Medium (DMEM), Penicillin Streptomycin Mixtures (Pen-Strep), and Trypsin-EDTA Solution (0.25%) were purchased from HyClone Laboratories, Inc. Normocin was purchased from Invivogen (San Diego, CA, USA).

Liquid-state ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using a Bruker AVANCE III HD 400 MHz NMR Spectrometer. Chemical shifts were reported as δ values relative to tetramethylsilane (TMS) as internal reference. MALDI-TOF mass spectra were recorded using a Bruker BIFLEX III Ultra-High-Resolution Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometer. Ultraviolet–visible (UV-vis) absorption spectra were recorded on a Shimadzu UV-2700 Double Beam UV-vis Spectrophotometer. Fourier transform infrared (FT-IR) spectra were obtained in the 4000~400 cm^{-1} range using a Thermo Scientific Nicolet iS50 FT-IR Spectrometer equipped with single reflection diamond ATR module. Elemental microanalyses (EA) were performed with an Elementar Vario EL Cube Elemental Analyzer. Scanning electron microscopy (SEM) micrographs were recorded on a Hitachi SU8010 Scanning Electron Microscope. Transmission electron microscope (TEM) micrographs were recorded on a Hitachi HT7700 120kV Compact-Digital Transmission Electron Microscope. Powder X-ray diffraction (PXRD) patterns were obtained on a Rigaku SmartLab SE X-Ray Powder Diffractometer with Cu Kα line focused radiation (λ=1.5405 Å) from 2θ=5.00° up to 50.00° with 0.01° increment. X-ray photoelectron spectroscopy (XPS) spectra were obtained on a Thermo Fisher Scientific ESCALAB 250Xi XPS System. Nitrogen isotherms were measured at 77 K using a Micromeritics ASAP2020 HD88 Surface Area and Porosity Analyser. Before measurement, the samples were degassed in vacuum at 120°C for 12 h. The specific surface area was calculated using the Brunauer–Emmett–Teller (BET) equation from the adsorption isotherm branch with a relative pressure range, p/p_0=0.05~0.2. The pore size distribution was determined from the adsorption isotherm data using the Horváth–Kawazoe (HK) method. Hydrodynamic particle size and zeta potential were measured using Malvern Zetasizer Nano ZS90 System. Flow cytometry was analyzed on a BD FACSVerse Flow Cytometer. Laser

scanning confocal fluorescence images were captured with a Leica TCS SP8 Confocal Laser Scanning Microscopy with an objective lens (×20, ×10). Glass bottom dishes were purchased from Cellvis (Mountain View, CA, USA). Microplate assays were carried out on a Molecular Devices SpectraMax i3x Multi-Mode Microplate Detection System.

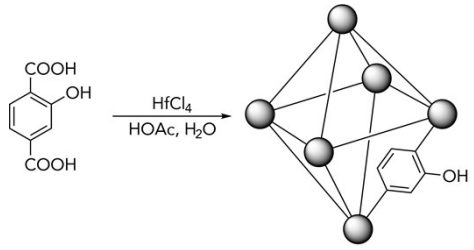
2. Cell Cultures and Laboratory Animals

The MCF-7 (human breast adenocarcinoma cell line) was provided by Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China), and cultured in DMEM supplemented with FBS (10%), Normocin (50 μg/mL), penicillin (100 U/mL) and streptomycin (100 μg/mL) in an atmosphere of 5% CO_2 and 95% air at 37°C.

Nude mice (BALB/c-nu, femina, aged 5 weeks, 15~20 g) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. Animal experiments were reviewed and approved by the Ethics Committee of Shandong Normal University (Jinan, China). All the animal operations complied with Chinese government relevant guidelines and regulations for the care and use of experimental animals.

MCF-7 cancer cells (5×10^6 cells) suspended in DPBS (50 μL) were subcutaneously injected into the flanks of each mice to establish MCF-7 xenograft model. Length (L) and width (W) of the tumor were determined by digital calipers. The tumor volume (V) was calculated by the formula: $V=1/2\times L\times W^2$. When the tumor size reached ~150 mm^3, animals were used in the experiments.

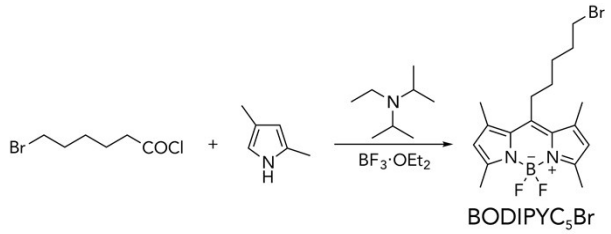
3. Synthesis of UiO-66-OH(Hf) (1)



Sch. S1 Synthesis of UiO-66-OH(Hf) (**1**)

As shown in Sch. S1, a mixture of $HfCl_4$ (336 mg, 1.05 mmol), 2-hydroxyterephthalic acid (182 mg, 1.0 mmol), acetic acid (4.0 mL) in water (6.0 mL) was heated at 100°C for 48 h. The resulting particles were isolated by centrifugation (12000 rpm for 30 min), and completely washed with ethanol. Then, the resulting solids were dried in air at 40°C to generate nanoscale UiO-66-OH(Hf) as milky white crystalline solids. FT-IR (ATR, cm^{-1}): 3253 (b), 2977 (m), 1699 (w), 1583 (s), 1500 (s), 1424 (vs), 1245 (m), 1158 (w), 1045 (w), 963 (w), 798 (w), 768 (m), 680 (m), 578 (w), 475 (w).

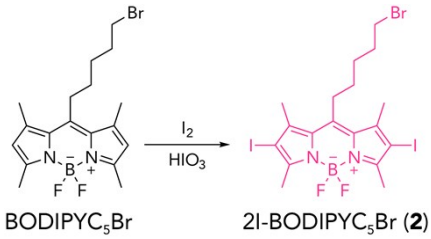
4. Synthesis of BODIPYC$_5$Br



Sch. S2 Synthesis of BODIPYC$_5$Br.

As shown in Sch. S2, in N_2, a mixture of 6-bromohexanoyl chloride (5.6 g, 26 mmol) and 2,4-dimethyl-1*H*-pyrrole (5.0 g, 52.5 mmol) in dichloromethane (200 mL) was refluxed for 1 h, and then allowed to cool to room temperature. Then, *N*,*N*-diisopropylethylamine (15 mL) was added and heated to reflux for 1 h. After addition of $BF_3{\cdot}Et_2O$ (15 mL), the mixture was refluxed for additional 3 h. After stirred overnight, the analytical pure product was obtained by neutral alumina column chromatography (eluant, dichloromethane). Yield: 4.7 g (46%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.06 (s, 2H), 3.44 (t, *J*=5.1 Hz, 2H), 2.99-2.94 (m, 2H), 2.52 (s, 6H), 2.42 (s, 6H), 1.95-1.89 (m, 2H), 1.69-1.63 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 153.90, 145.90, 140.25, 131.36, 121.66, 33.38, 32.18, 30.88, 28.55, 28.19, 16.39, 14.44. MALDI-TOF MS, Calcd. For [M], 396.118, Found, 396.386. UV-vis (EtOH), 497 nm. FT-IR (ATR, cm^{-1}): 2927 (m), 2868 (w), 1550 (vs), 1509 (vs), 1475 (s), 1440 (m), 1409 (m), 1372 (m), 1340 (w), 1307 (m), 1270 (m), 1252 (w), 1224 (m), 1200 (vs), 1157 (m), 1107 (m), 1080 (s), 1070 (s), 1028 (m), 986 (s), 837 (w), 819 (w), 798 (w), 716 (w), 644 (vw), 626 (w), 583 (w), 554 (vw), 482 (w). Anal. Calcd. For $C_{18}H_{24}BBrF_2N_2$ (%): C, 54.44; H, 6.09; N, 7.05, Found: C, 54.14; H, 5.91; N, 7.32.

5. Synthesis of 2I-BODIPYC$_5$Br (2)

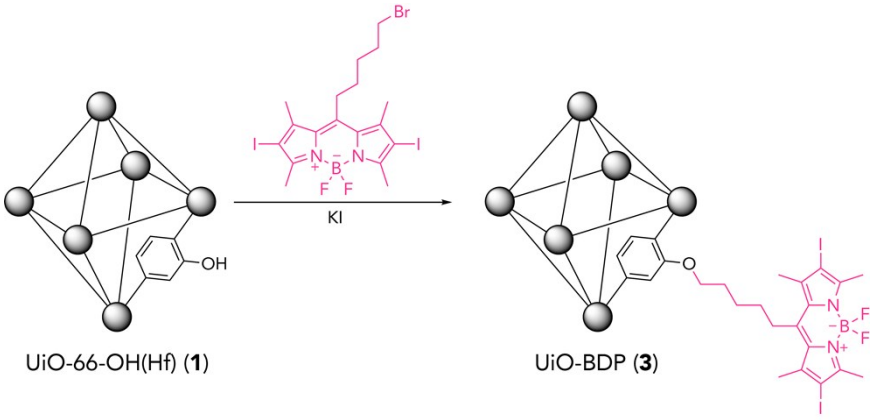


Sch. S3 Synthesis of 2I-BODIPYC$_5$Br (**2**).

As shown in Sch. S3, a mixture of BODIPYC$_5$Br (1.0 g, 2.5 mmol) and iodine (1.6 g, 6.25 mmol), and iodic acid aqueous solution (1.0 mL, 0.9 g/mL) in ethanol (400 mL) was refluxed for 30 min. After cooling to room temperature, the product was purified by neutral alumina column chromatography (eluant, dichloromethane/hexane, v/v=1:1) to afford **2** as the orange red solids. Yield: 1.1 g (69%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 3.44 (t, *J*=6.5 Hz, 2H), 3.07-2.99 (m, 2H), 2.62 (s, 6H), 2.49 (s, 6H), 1.93 (p, *J*=6.5 Hz, 2H), 1.73-1.60 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 155.41, 145.54, 142.15, 131.31, 86.56, 33.21, 32.07, 30.64, 29.03, 28.43, 19.00, 16.13. MALDI-TOF MS, Calcd. For [M], 647.912, Found, 647.557. UV-vis (EtOH), 527 nm. FT-IR (ATR, cm^{-1}): 2925 (m), 2859 (w), 1538 (vs), 1462 (m), 1394 (m), 1347 (m), 1306 (w), 1272 (w), 1252 (w), 1207 (w), 1186 (s), 1146 (m), 1077 (m), 994 (m), 753 (w), 719 (w), 587 (w), 527 (w). Anal. Calcd. For $C_{18}H_{22}BBrF_2I_2N_2$ (%): C, 33.32; H, 3.42; N, 4.32, Found: C, 33.48; H, 3.21; N, 4.55.

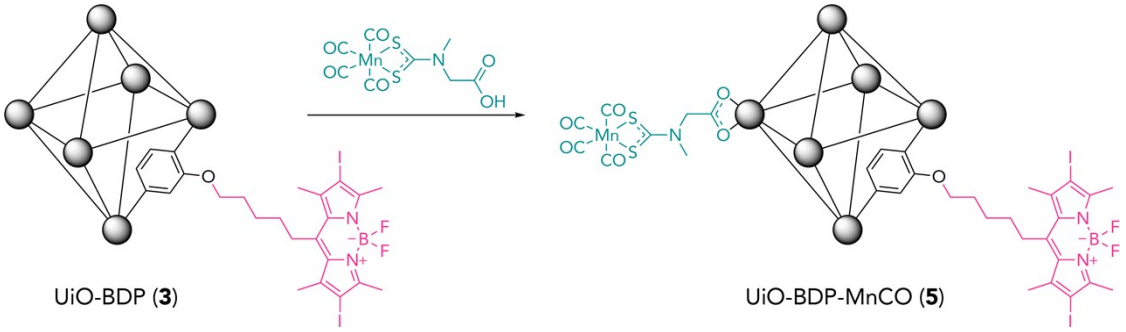
6. Synthesis of UiO-BDP (3)

As shown in Sch. S4, a mixture of **1** (5.0 mg), **2** (5.0 mg, 7.7 μmol), KI (1.0 mg, 6.0 μmol) in DMF (5.0 mL) was stirred for 72 h at room temperature. The resulting solids were isolated by centrifugation (12000 rpm for 30 min) and completely washed with DMF and water. The obtained pink **3** was resuspended in water and stored at 4°C for use. FT-IR (ATR, cm-1): 3233 (b), 2976 (m), 1700 (w), 1584 (s), 1500 (s), 1424 (vs), 1245 (m), 1158 (w), 1099 (w), 1044 (w), 963 (w), 833 (w), 798 (w), 768 (m), 592 (w), 576 (w), 527 (vw), 475 (w).



Sch. S4 Synthesis of UiO-BDP (**3**).

7. Synthesis of UiO-BDP-MnCO (5)



Sch. S5 Synthesis of UiO-BDP-MnCO (**5**).

As shown in Sch. S5, a mixture of **3** (1.0 mg), **4** (2.0 mg, 6.0 μmol) in water (10.0 mL) was stirred for 4 h at 4°C. Then, the particles were isolated by centrifugation (12000 rpm for 30 min) and was completely washed with water. The obtained pink **5** was resuspended in water and stored at 4°C for use. FT-IR (ATR, cm^{-1}): 3252 (b), 2087 (m), 2006 (vs), 1990 (vs), 1968 (vs), 1938 (vs), 1717 (m), 1582 (s), 1501 (s), 1424 (vs), 1397 (vs), 1281 (w), 1245 (m), 1217 (m), 1100 (w), 1027 (w), 998 (w), 798 (w), 769 (w), 662 (m), 619 (m), 478 (w), 441 (w).

8. 2I-BODIPY and MnCO Contents

The contents of 2I-BODIPY and MnCO in **3** and **5** were determined by ICP-OES. Specifically, the sample was dissolved in the mixed acid (H_2SO_4/HNO_3, v/v=1:1), and the solution was diluted with water, and the contents of B and Mn were determined.

The final data was based on the results of 3 independent experiments. The chemical composition of $Hf_6O_4(OH)_4(HPTA)_{6-y/2}(2I\text{-}BODIPY)_x(MnCO)_y$ for **3** or **5** was obtained by the following equations based on ICP-OES results:

$$\begin{cases} \omega(B) = \dfrac{10.81 * x}{6 * 178.49 + 4 * 16.00 + 4 * 17.01 + \left(6 - \dfrac{y}{2}\right) * 180.12 + x * 568.00 + y * 330.19} * 10^3 \\ \omega(Mn) = \dfrac{54.94 * y}{6 * 178.49 + 4 * 16.00 + 4 * 17.01 + \left(6 - \dfrac{y}{2}\right) * 180.12 + x * 568.00 + y * 330.19} * 10^3 \end{cases}$$

The unknown numbers x and y were obtained by substituting ω(B) and ω(Mn) in the above equations.

Tab. S1 2I-BODIPY Content in **3**.

Content	Unit	#1	#2	#3	MEAN	SD
ω(B)	mg/g	1.460	1.350	1.620	1.477	0.136
m(2I-BODIPY)	μmol/mg	0.135	0.125	0.150	0.137	0.013
x	–	0.334	0.307	0.374	0.338	0.034

Tab. S2 2I-BODIPY and MnCO Contents in **5**.

Content	Unit	#1	#2	#3	MEAN	SD
ω(B)	mg/g	1.100	0.880	1.040	1.007	0.114
m(2I-BODIPY)	μmol/mg	0.102	0.081	0.096	0.093	0.011
ω(Mn)	mg/g	58.700	61.100	63.540	61.113	2.420
m(MnCO)	μmol/mg	1.068	1.112	1.157	1.112	0.044
x	–	0.339	0.271	0.329	0.313	0.037
y	–	3.559	3.699	3.956	3.738	0.202
6-y/2	–	3.882	3.880	3.693	3.818	0.109

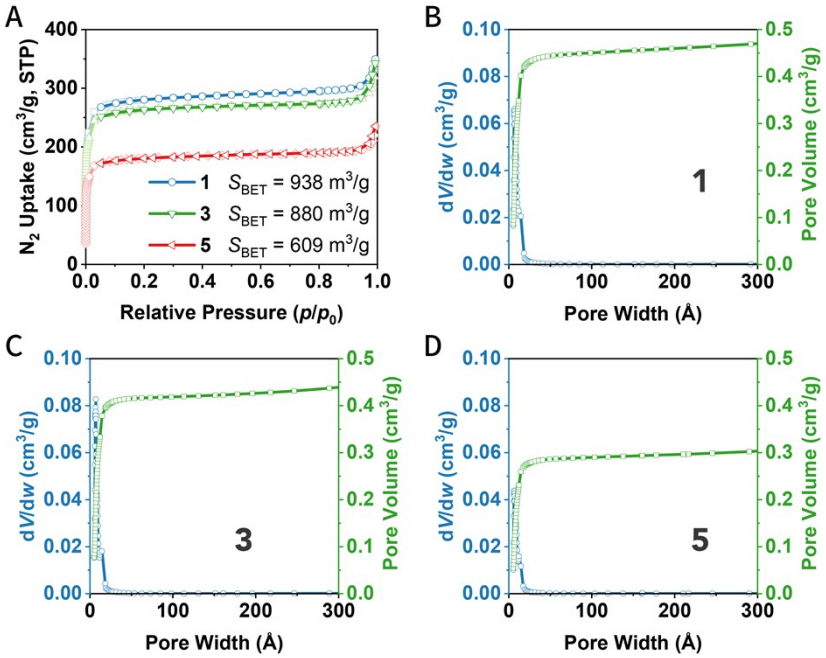


Fig. S1 (A) N_2 adsorption-desorption isotherms (77 K) of **1**, **3**, and **5**. (B-D) Pore size distribution and cumulative pore volume profiles of **1** (B), **3** (C), and **5** (D).

9. FT-IR Spectra and UV-Vis Absorption Spectra

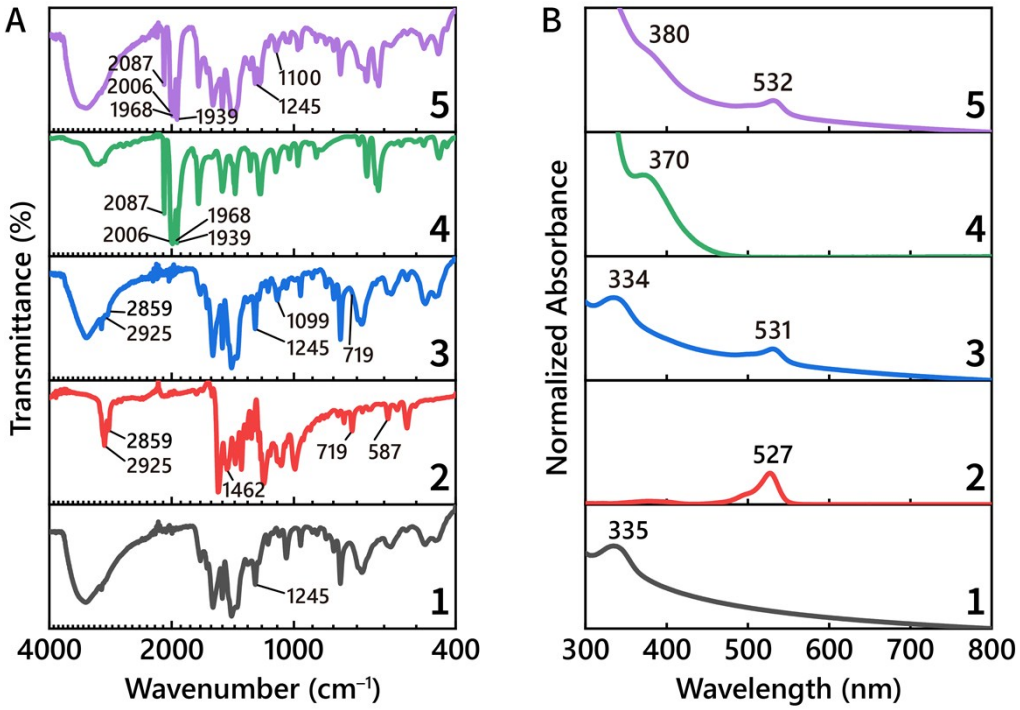


Fig. S2 (A) FT-IR spectra of **1**, **2**, **3**, **4**, and **5**. (B) UV-vis absorption spectra of **1**, **2**, **3**, **4**, and **5**.

The FT-IR indicated that the methylene group bands at 2925, 2859, 1462 and 719 cm^{-1} belonging to **2** were also observed in **3** and **5**. Meanwhile, the C–Br stretching vibration of **2** at 587 cm^{-1} disappeared in **3** and **5**, implying that **2** was covalently attached to **1**. Additional convincing evidence was that the Ph–O–C stretching vibrations at 1099 and 1100 cm^{-1} were also detected in **3** and **5**, further confirming the presence of the 2I-BODIPY species. On the other hand, the –OH vibration at 1245 cm^{-1} in **1** still appeared in **3** and **5**, suggesting that the PSM reaction was not quantitative. The CO stretching vibrations at 2087, 2006, 1968, 1939 cm^{-1} of **4** were clearly observed in **5**, demonstrating successful MnCO decoration.

The absorption peaks at 335 nm for **1** and at 527 nm for **2** both appeared in **3** at 334 and 531 nm, and the absorption peak at 370 nm for **4** appeared in **5** at 380 nm.

10. Controlled Experiments of Material Structures

In order to further verify that **2** and **4** were covalently bonded to the framework of **1**, a control experiment was designed and conducted. Specifically, **3** and **5** were dispersed in an ethanol, followed by centrifugation, and the UV-vis spectra of the supernatant was measured. As controls, the mixtures of **1**+**2** and **1**+**2**+**4** were dispersed in an ethanol, followed by centrifugation, and the UV-vis spectra of the supernatant was measured.

Due to the high solubility of **2** and **4** in ethanol, simple physical mixing allowed **2** and **4** to be easily extracted in ethanol, resulting in a UV-vis spectrum of the supernatant showing the characteristic absorption peaks of **2** and **4** (Fig. S3A and S3B). However, in **3** and **5**, the 2I-BODIPY and MnCO moieties were covalently bonded to **1**, and they could not be extracted by ethanol, so no their characteristic absorption peaks were observed (Fig. S3C and S3D).

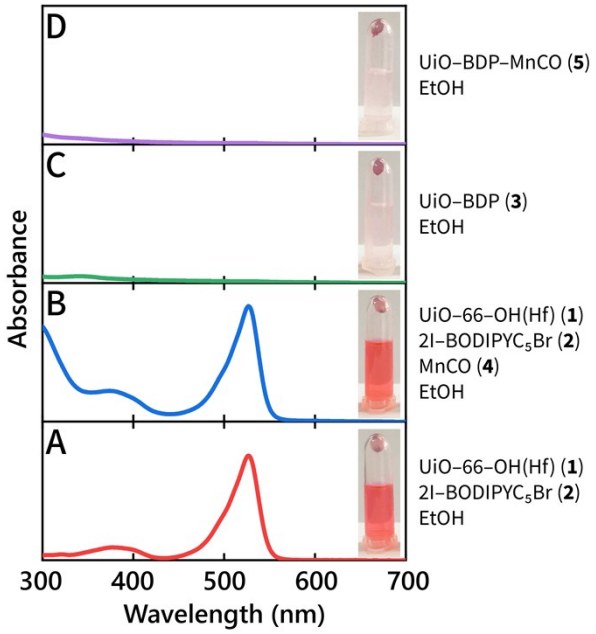


Fig. S3 Control experiments confirming the 2I-BODIPY and MnCO moieties to be covalently bonded to **1**. Specifically, **1**+**2** (A), **1**+**2**+**4** (B), **3** (C), **5** (D) were dispersed in ethanol, after centrifugation, the UV-vis spectra of the supernatants were measured.

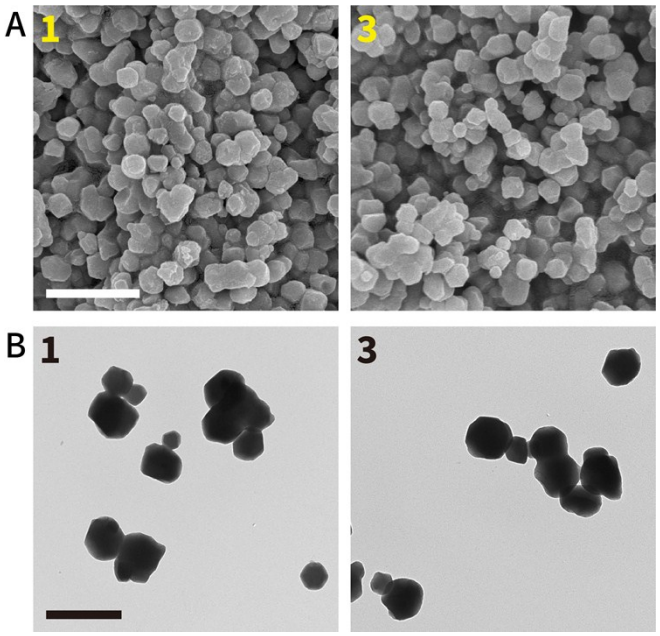


Fig. S4 (A) SEM images of **1** and **3**. Scale bar, 1 μm. (B) TEM images of **1** and **3**. Scale bar, 500 nm.

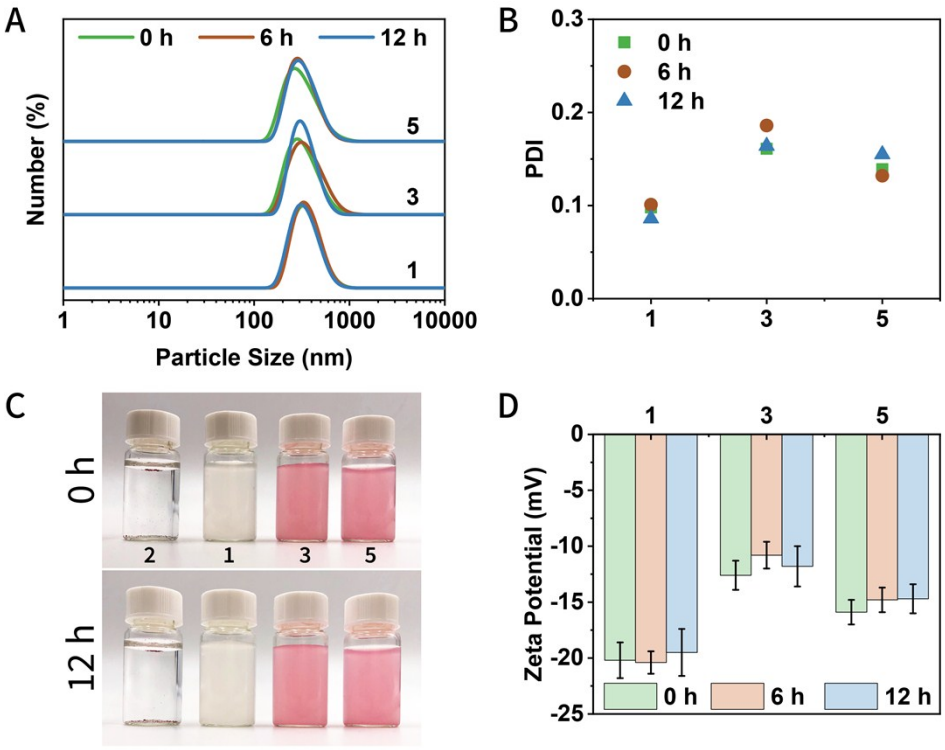


Fig. S5 (A) DLS size distributions of **1**, **3**, and **5** in PBS (pH = 6.5) at different times. (B) Particle dispersion index (PDI) of **1**, **3**, and **5** in PBS (pH = 6.5) at different times. (C) Digital photographs of **1**, **3**, and **5** PBS dispersions at different times. (D) Zeta potentials of **1**, **3**, and **5** in PBS. Data are presented as the mean±SD (n = 3).

11. Singlet Oxygen Generation Experiment

DMF dispersions of **1**, **3** and **5** (2 mL, 5.0 μM, 2I-BODIPY equiv) were pipetted into quartz cuvette, and DPBF DMF solution (100 μL, 1 mM) was added. Then the mixture was exposed to green LED (20 mW/cm^2) at room temperature for 120 s. The absorbance of DPBF at 414 nm in the mixture was recorded at 20 s intervals. The $^1O_2$ generation rate was determined from the reduced the absorbance over time. The same dispersion without DPBF was used as the reference for UV-vis measurement.

To characterize the difference in the rate of $^1O_2$ produced by different samples, the ratios A/A_0 of absorbance A and the initial absorbance A_0 at 414 nm at different irradiation times were calculated and plotted as the ordinate for the irradiation time. DPBF solution without adding **1**, **3** or **5** was used as a control (CG).

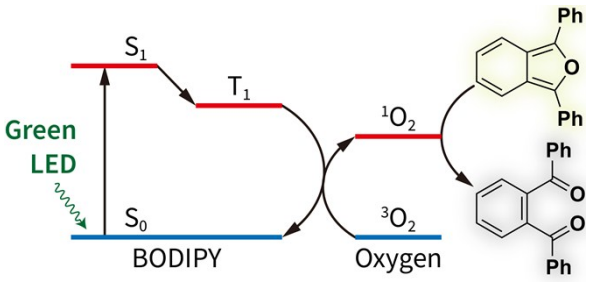


Fig. S6 Schematic diagram of 2I-BODIPY-induced $^1O_2$ generation.

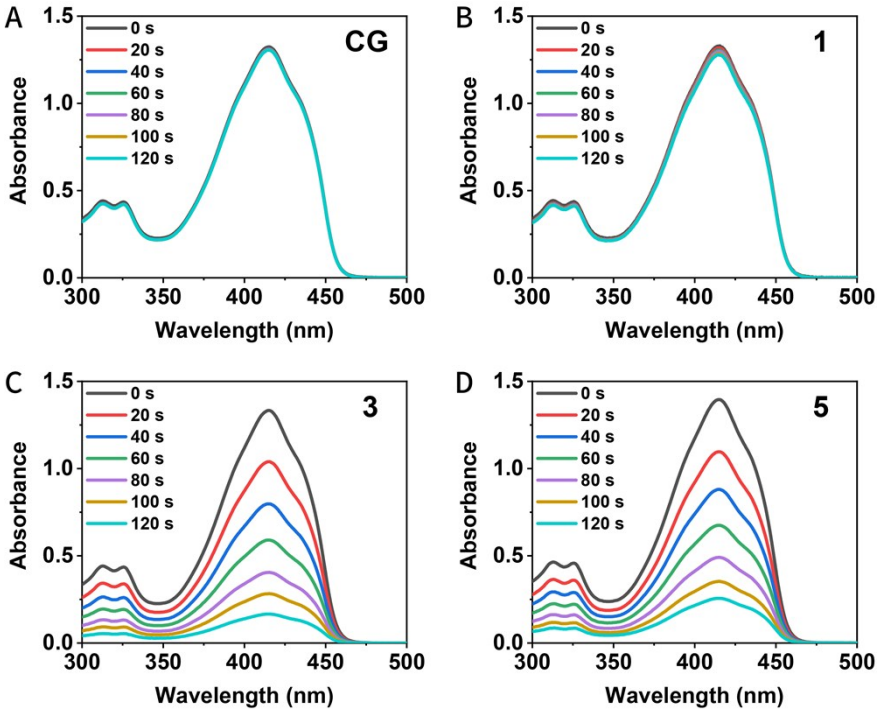


Fig. S7 DPBF UV-vis spectra of the singlet oxygen generation experiments for control group (A), **1** (B), **3** (C), and **5** (D).

12. Carbon Monoxide Generation Experiment

To assess ROS-induced CO release, H_2O_2 solution (100 μL, 0~1 M) was injected into an airtight 3-necked flask containing PBS dispersion of **5** (10 mL, 1.0 mM, MnCO equiv) and stirred for 2 h under the green LED (0 or 20 mW/cm^2). Subsequently, the gas above the dispersion was collected with a syringe, and then the CO concentration was measured with CO gas detector tubes. The experiment was independently repeated three times, and the CO concentration was expressed as a function of the actual H_2O_2 concentration in reaction system. For the group with H_2O_2 end concentration of 1 mM, the solution after the reaction was centrifuged, and the solid was taken for XPS spectrometry.

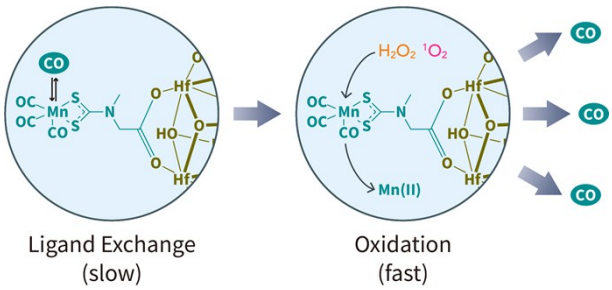


Fig. S8 Schematic diagram of MnCO-induced CO release.

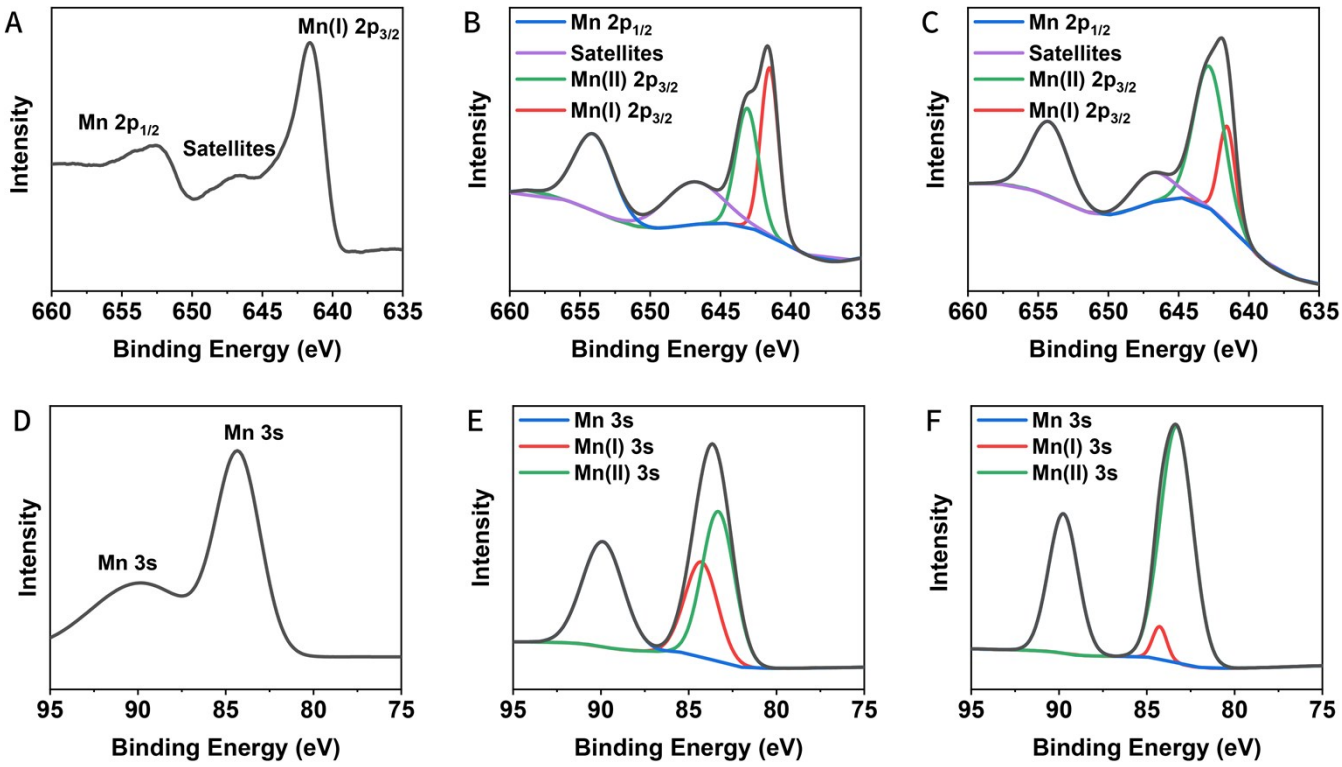


Fig. S9 XPS spectra of Mn 2p and 3s. (A, D) XPS spectra of **5**. (B, E) XPS spectra of **5** after stirring for 2 h in H_2O_2 solution (1 mM). (C, F) XPS spectra of **5** after stirring for 2 h in H_2O_2 solution (1 mM) under the green LED (20 mW/cm^2).

13. Flow Cytometry Analysis of Cell Uptake

Cells were seeded into 6-well plates with a cell number of ~200k cells/well and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were subjected to different pre-treatments as follow: (i) DPBS, CO_2 incubator, 37°C, 1 h; (ii) HBSS, air atmosphere, 4°C, 1 h; (iii) CPZ (20 μg/mL), CO_2 incubator, 1 h; (iv) MβCD (10 mg/mL), CO_2 incubator, 1 h; (v) AMR (75 μg/mL), CO_2 incubator, 1 h. After these different pre-treatments, the cells were incubated with dispersion of **5** (2 mL, 0.5 μM, 2I-BODIPY equiv) in 4°C or 37°C for 4 h. The cells were washed with DPBS twice carefully, collected by using trypsin-EDTA (0.25%), and used for flow cytometry study.

14. ICP-OES Analysis of Cell Uptake

Cells were seeded into 6-well plates and grown to confluence in a CO_2 incubator. After removal of the culture medium, the cells were subjected to different pre-treatments as follow: (i) DPBS, CO_2 incubator, 37°C, 1 h; (ii) HBSS, air atmosphere, 4°C, 1 h; (iii) CPZ (20 μg/mL), CO_2 incubator, 1 h; (iv) MβCD (10 mg/mL), CO_2 incubator, 1 h; (v) AMR (75 μg/mL), CO_2 incubator, 1 h. After these different pre-treatments, the cells were incubated with DPBS dispersion of **5** (2 mL, 100 μg/mL) in 4°C or 37°C for 4 h. The cells were washed with DPBS twice carefully, and were lysed in the presence of Triton X-100 (1%). The lysate was collected, digested with mixed acid (H_2SO_4/HNO_3, v/v=1:1), diluted with water, and filtered through a filter (0.22 μm). The Hf contents in the clear liquid were determined by ICP-OES.

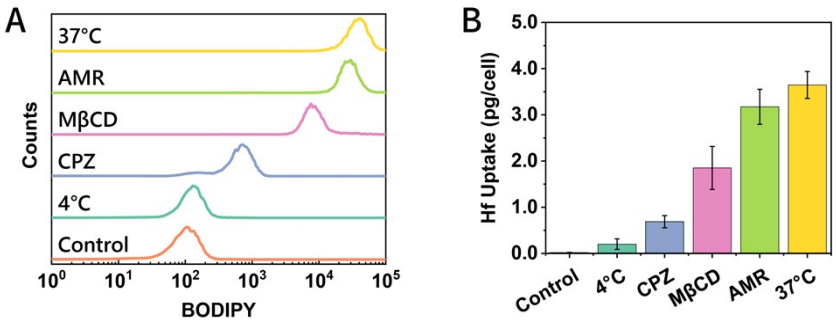


Fig. S10 Mechanism of cellular uptake in MCF-7 cells studied by flow cytometry analysis (A) and intracellular Hf determination (B). The cells were pretreated with 4°C (energy-dependent endocytosis inhibitor), chlorpromazine hydrochloride (CPZ, clathrin-dependent endocytosis inhibitor), methyl-β-cyclodextrin (MβCD, caveolin-dependent endocytosis inhibitor), and amiloride hydrochloride (AMR, micropinocytosis inhibitor). After these different pretreatments, the cells were incubated with a dispersion of 5 at 4°C or 37°C, and the relative contents of nanoparticles in the cells were assessed.

15. Intracellular Singlet Oxygen Measurement

Cells were seeded into glass bottom dishes and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **2, 3, 4,** or **5** (200 μL; 0.5 μM, 2I-BODIPY equiv; 6.0 μM, MnCO equiv) in a CO_2 incubator for 4 h, washed with DPBS twice, and further incubated with SOSG (5 μM, 200 μL) for 15 min. The cells were exposed to green LED (20 mW/cm^2) for different times and imaged with a laser scanning confocal microscope. The green images were excited by 488 nm light, and the emission wavelength range was collected at 525±20 nm. The mean fluorescence intensity (MFI) was analyzed by ImageJ software.

16. Intracellular CO Measurement

Cells were seeded into glass bottom dishes and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **3** or **5** (200 μL; 0.5 μM, 2I-BODIPY equiv; 6.0 μM, MnCO equiv) in a CO_2 incubator for 4 h, and washed with DPBS twice. Then, the cells were exposed to green LED (20 mW/cm^2) for different times. After additional 4 h incubation, the cells were incubated with NR-PdA (200 μL, 2 μM) for 30 min, and washed with DPBS twice. Next, the laser scanning confocal fluorescence images were captured. The red images were excited by 561 nm light, and the emission wavelength range was collected at 595±25 nm. The MFI was analyzed by ImageJ software.

17. Mitochondrial Membrane Potential

Cells were seeded into glass bottom dishes and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **3** or **5** (200 μL; 0.5 μM, 2I-BODIPY equiv; 6.0 μM, MnCO equiv) in a CO_2 incubator for 4 h, and washed with DPBS twice. Then, the cells were exposed to green LED (20 mW/cm^2) for 5 min. The cells without green LED irradiation were used as control. After additional 4 h incubation, the cells were incubated with JC-1 (200 μL, 10 μg/mL) for 10 min, and washed with DPBS twice. Next, the laser scanning confocal fluorescence images were captured. The green images of monomer were excited by 488 nm light, and the emission wavelength range was collected at 530±15 nm. The red images of *J*-aggregate were excited by 514 nm light, and the emission wavelength range was collected at 590±17 nm. The MFI was analyzed by ImageJ software.

18. Lactate Measurement

Cells were seeded into 6-well plates with a cell number of ~100k cells/well and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DMEM dispersion of **2**, **3**, **4**, and **5** (2 mL; 10 or 40 μM, MnCO equiv.; 0.84 or 3.4 μM, 2I-BODIPY equiv) in a CO_2 incubator for 24 h, and washed with DPBS twice. The cells without treatment were used as control. Next, the cell media were collected and centrifuged to remove excess nanoparticles for extracellular lactate measurements; the cells were lysed in the presence of Triton X-100 (1%) at 4°C for intracellular lactate measurements. The lactate measurements were used a Lactate Assay Kit according to the protocol provided by the kit supplier.

19. ATP Measurement

Cells were seeded into 6-well plates with a cell number of ~100k cells/well and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DMEM dispersion of **2**, **3**, **4**, and **5** (2 mL; 10 or 40 μM, MnCO equiv; 0.84 or 3.4 μM, 2I-BODIPY equiv) in a CO_2 incubator for 24 h, and washed with DPBS twice. The cells without treatment were used as control. Next, the cells were lysed in the presence of Triton X-100 (1%) at 4°C for intracellular ATP measurements. The ATP measurements were used an ATP Assay Kit according to the protocol provided by the kit supplier.

20. *In Vitro* PDT Experiment

Cells were seeded into 96-well plates with a cell number of ~5k cells/well and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **3** or **5** (100 μL; 0~5.0 μM, 2I-BODIPY equiv; 0~59.7 μM, MnCO equiv) in a CO_2 incubator for 4 h, and washed with DPBS twice. Then, the cells were exposed to green LED (20 mW/cm^2) for different time. After additional 24 h incubation, MTT (10 μL, 5 mg/mL) was added to each well and incubated for additional 4 h in a CO_2 incubator. Finally, the supernatants were removed and DMSO (100 μL) was added into each well, followed by recording the absorbance at 490 nm.

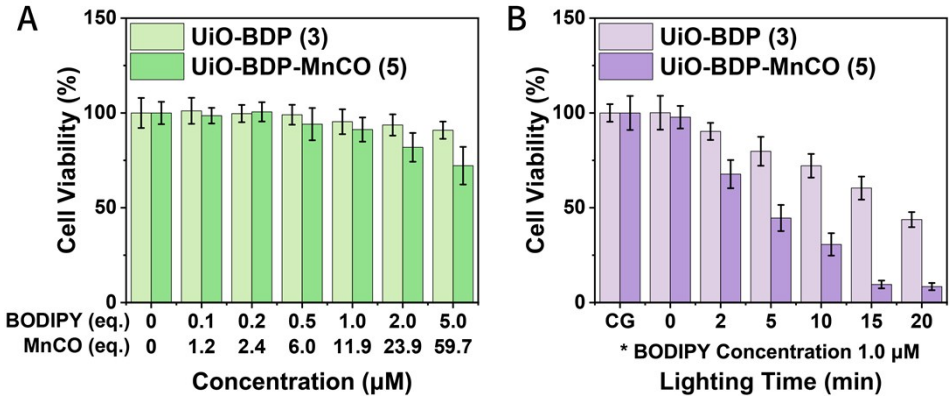


Fig. S11 (A) Dark toxicity of **3** and **5**. (B) Light-dependent cytotoxicity of **3** and **5** (1.0 μM, 2I-BODIPY equiv). Data were presented as the mean±SD (n = 5).

21. Calcein-AM/PI Double Staining

Cells were seeded into culture dishes and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **3** or **5** (500 μL, 2.0 μM, 2I-BODIPY equiv) in CO_2 incubator for 4 h, and washed with DPBS twice. Then, the cells were exposed to green LED (20 mW/cm^2) for 0 or 10 min. After additional 4 h incubation, the cells were collected using Trypsin-EDTA Solution (0.25%), washed with DPBS twice carefully, and were stained with Calcein-AM (500 μL, 2 μM) and PI (500 μL, 4.5 μM) for 15 min.

Finally, the cells were washed with DPBS twice carefully, and imaged with a laser scanning confocal microscope. The green images of living cells were excited by 488 nm light, and the emission wavelength range was collected at 520±20 nm. The red images of dead cells were excited by 514 nm light, and the emission wavelength range was collected at 640±20 nm.

22. *In Vivo* PDT Experiment

The nude mice bearing MCF-7 tumors (n=30) were randomly distributed into 6 groups, and injected and illuminated according to the specified procedure. The tumor volume and nude mouse body weight were recorded daily during the experimental period.

Table S3. Treatment plan of *in vivo* PDT experiments.

Group	Injection	Volume	2I-BODIPY (equiv)	MnCO (equiv)	Green Laser
i	DPBS	50 μL	—	—	—
ii	**2&4**	50 μL	75 μM	0.90 mM	0.5 W/cm^2, 10 min
iii	**3**	50 μL	75 μM	—	—
iv	**5**	50 μL	75 μM	0.90 mM	—
v	**3**	50 μL	75 μM	—	0.5 W/cm^2, 10 min
vi	**5**	50 μL	75 μM	0.90 mM	0.5 W/cm^2, 10 min

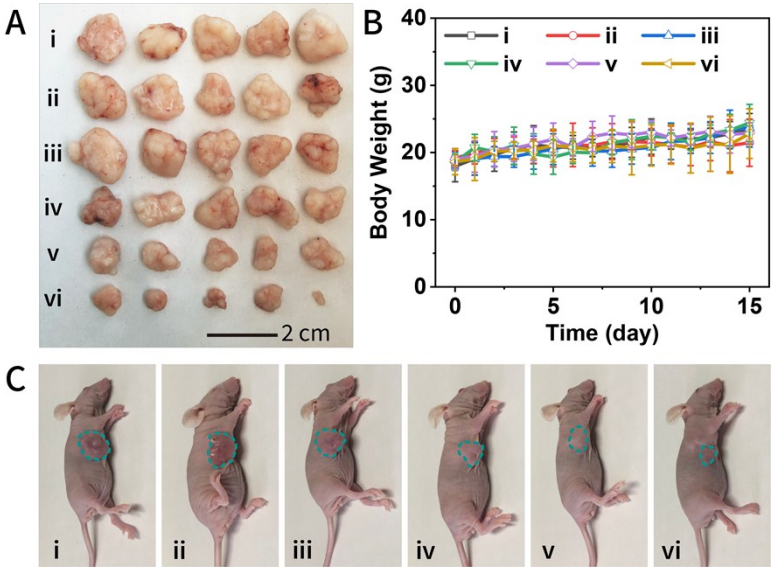


Fig. S12 (A) Photographs of tumors obtained after treatment. Scale bar: 2 cm. (B) Body weight of the mice in various groups during the treatment. Data are presented as the mean±SD (n = 5). (C) Representative photographs of MCF-7 tumor-bearing nude mice at the end of the treatment.